THE LIFE HISTORY IN CULTURE OF *PLEONOSPORIUM CARIBAEUM* (CERAMIACEAE, RHODOPHYTA) FROM THE CARIBBEAN

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ABSTRACT - *Pleonosporium caribaeum* (Børgesen) Norris demonstrates a *Polysiphonia*-type life history in culture. The life history was completed in 50 to 60 days with the tetrasporophytic plants requiring 35-45 days to mature. Mature tetrasporophytes produced both sessile tetrasporangia and sessile as well as pedicellate polysporangia. Both tetrasporangia and polysporangia gave rise to male and female gametophytes, showing a 1:1 sexual segregation. Spermatangial branches were observed which appeared to be both stalked and sessile. This condition is recognized as simply reflecting the degree of development of the individual branch.

RÉSUMÉ - *Pleonosporium caribaeum* (Børgesen) Norris présente un cycle de type-*Polysiphonia*. Le cycle a été réalisé entre 50 et 60 jours, les tétrasporophytes nécessitant 35 à 45 jours pour arriver à maturité. Les tétrasporophytes fertiles ont produit des tétrasporocystes sessiles ainsi que des polysporocystes sessiles ou pédicellés. Tétrasporocystes et polysporocystes ont donné naissance à des gamétophytes mâles et femelles, avec ségragation sexuelle de 1:1. La structure des rameaux mâles paraissait être sessile ou pédicellée selon le degré de développement de la cellule sous-jacente. (traduit par la Rédaction)

KEY WORDS : life history, development, culture, reproduction, *Pleonosporium caribaeum*, Ceramiaceae, Rhodophyta.

INTRODUCTION

Pleonosporium caribaeum (Børgesen) Norris was originally described as a species of the newly created genus *Mesothamnion* (Børgesen, 1917). At that time *Mesothamnion* was considered to be separate from *Pleonosporium* by possessing uninucleate cells, alternate and radially arranged branches, tetrasporic meiosporangia, "stalked" spermatangial fascicles and subterminal procarps (Børgesen, 1917; Kylin, 1956). Further studies on *Mesothamnion* and *Pleonosporium* (Itono, 1977; Ardré *et al.*, 1982), including the description of new species with intermediate characteristics, have blurred the distinction between the genera. Ardré *et al.* (1982) further demonstrated in an examination of the type specimen that *Mesothamnion* cells are actually plurinucleate. They also discovered what they claimed were the presence of both sessile and stalked spermatangial fascicles on the type specimen. Norris (1985) finally concluded after an evaluation of the female reproductive structures of both genera that there are no dis-

tinguishing characters sufficient to maintain *Mesothamnion* as a distinct genus from *Pleonosporium*.

Norris (1985) studying with South African specimens and Kim & Lee (1988) working with Korean plants examined the reproduction morphology of *Pleonosporium caribeaum*. Kim & Lee (1988) further cultured the species through its life history. In this paper we examine *Pleonosporium caribaeum* from the Caribbean and review its life history in culture as well as its vegetative and reproductive morphology.

MATERIALS AND METHODS

Pleonosporium caribaeum was collected subtidally at depths between 17 and 24 m in an algal plain 1.5 km seaward of Media Luna Reef and 1.6 km seaward of Margarita Reef at La Parguera, Puerto Rico. Plants collected by Scuba were transported to the laboratory in seawater filled polyethylene bags. Specimens for morphological studies were preserved in 10% formalin in seawater immediately after collection. Whole mount slides were prepared of specimens stained with acidified 1% aniline blue in 20% Karo syrup. Line drawings were made with the aid of a camera lucida. Specimens for cytological examination were fixed immediately following return to the laboratory in a 2:1 mixture of ethanol and acetic acid diluted to 50% with seawater and stained with aceto-iron-haematoxylin-chloral hydrate (Wittmann, 1963).

Unialgal cultures were established by isolation of discharged carpospores. These were grown in polystyrene petri dishes containing 20 ml of 10% strength Provasoli ES medium (McLachlan, 1973) in sterile seawater with salinity adjusted to 35 ppt. Diatom growth was controlled with GeO_2 (5 mg l^{-1}) (Lewin, 1966) and cyanobacteria and bacteria controlled with Penicillin-G. Cultures were maintained at 25°C with photon fluence rates of 8-11 μmol $m^{-2}s^{-1}$ at 16:8 h (L:D) photoregimes. Tetrad analyses were conducted as described by Rueness and Rueness (1982).

RESULTS

Vegetative Structure

Plants are red to brick red in color and reach approximately 6 cm in height. Branching is to many orders and is alternately radial throughout with a rotation of 33%. The axes are ecorticate except for a few loose rhizoidal filaments arising from proximal cells of branches of the first order. Plants are attached to the substrate by these descending branched rhizoids and by multicellular discoid holdfasts arising from the lowermost axial cells. Thalli generally have one percurrent main axis, but often many subsequal branches are present. Cells of the main axes measure 175 to 225 μm in diameter, and are 1 to 1.5 diameters long in the basal portions. Axial cells become narrower and more elongate toward the middle portions, measuring 130 to 150 μm in diameter, and are 4(-6) diameters long. The apical cells of the main axes measure 6-8 μm in diameter and are 14-20 μm long. Apices are commonly overtopped by markedly incurved lateral branchlets (Fig. 1). Cells of the ultimate branches are 16-25 μm in diameter and measure 45-90 μm in length. All cells are multinucleate.

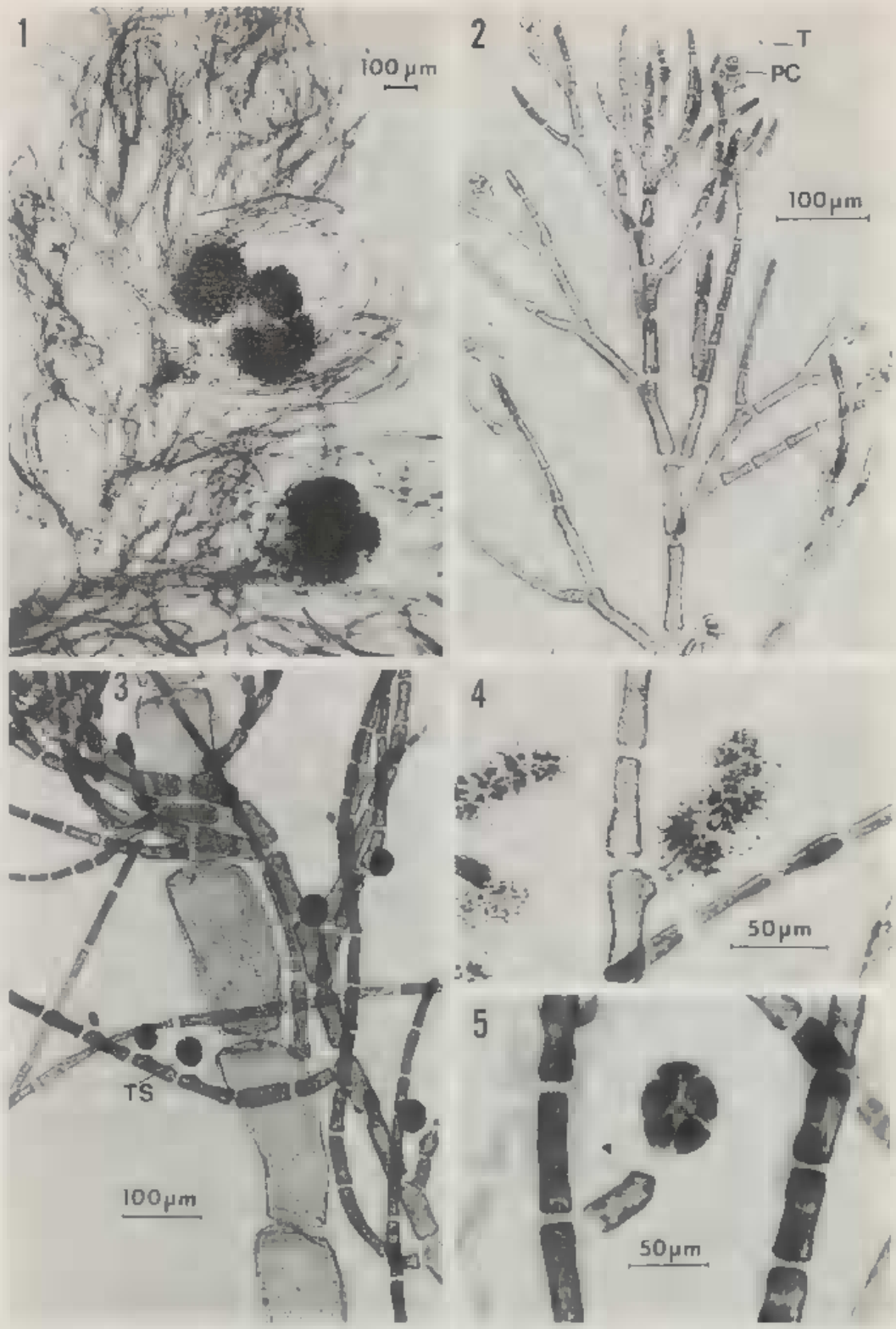

Fig. 1-5: *Pleonosporium caribaeum*. Fig. 1. General habit of a field collected carposporangial thallus. Fig. 2. Cultured female gametophyte bearing terminal procarps. Fig. 3. Seriate rounded tetrasporangia. Fig. 4. Spermatangial fascicle. Fig. 5. Pedicellate polysporangia with a second sporangial primordium (arrow). PC = procarp; T = trichogyne; TS = tetrasporangium.

Reproduction

Gametangial plants are dioecious. Procarp and carposporophyte development are as described by Ardré *et al.* (1982) and Norris (1985). Procarps are formed subterminally on determinate laterals (Fig. 2) from a procarp mother cell. The fertile subapical cell cuts off two periaxial cells, with the second formed becoming the support cell. Formation of the first periaxial cell normally results in the lateral displacement of the apical cell (Kylin, 1925; Norris, 1985) towards the side of the lateral branch borne on the hypogenous cell (Ardré *et al.*, 1982). The support cell then cuts off a sterile cell terminally and subsequently a four-celled carpogonial branch which is slightly arched with its convex face towards the apical cell (Figs. 8, 9). The trichogyne measures up to 80 μm in length and has a bulbous base (Fig. 9). Following fertilization, the trichogyne withers and the support cell cuts off an auxilliary cell (Fig. 10). The diploid nucleus is transferred to the auxiliary cell by direct fusion with the carpogonium. The diploidized auxiliary cells cuts off three or four gonimoblast initials from which the rounded gonimolobes develop (Fig. 11). As development of the gonimoboles continues, the auxiliary and the support cell form a large fusion cell. A degree of fusion also takes place between the subapical cell, the periaxial cell and the apical cell. As development of the carposporophyte proceeds, the hypogenous cell produces 4-6 involucral filaments which surround the carposporophyte (Fig. 11).

The spermatangial fascicles are specialized lateral uniseriate branches (Fig. 4). A spermatangial branch initial divides transversely 3 to 4 times to form the 4 to 5-celled fertile male axis (Fig. 6a-c). The basal cells measure 16.5 to 43.5 μm long and 9.3 to 15.3 μm in diameter. Cells of this fertile branch divide to form 4 periaxial cells which divide repeatedly to produce spermatangial mother cells (Fig. 6c). Two or three spermatangia are formed from each mother cell (Fig. 7). Depending on whether or not spermatangia are cut off on the basal-most branch cell, the fascicles appear to be sessile or pedicellate. The most commonly seen situation is that the basal-most cell of the spermatangial branch cuts off spermatangia and subsequently elongates. This results in a spermatangial fascicle that appears to be pedicellate (Fig. 4). Spermatangial fascicles occur singly and are located adaxially on the last two orders of branching. The clusters are 30-40 μm in diameter and 65-75 μm long.

Tetrasporangial plants produce both tetrasporangia and polysporangia, the former being more abundant. Both types of sporangia are produced adaxially in secund series (Fig. 3). The tetrasporangia are sessile, with spores tetrahedrally arranged. They are generally rounded to obovate and average 38.0 ± 4.7 SD μm in diameter (n = 10). Polysporangia are sessile or pedicellate on one or rarely two-celled pedicels (Fig. 5). A secondary polysporangium primordium sometimes develops adaxially from the pedicel (Fig. 5). Polysporangia average 43.9 ± 2.9 SD μm (n = 10) in diameter and contain 8-12 spores.

Life history and Reproduction in Culture

The life history of *Pleonosporium caribaeum* was completed in culture through several successive generations and a *Polysiphonia*-type life history was observed. Cultures were established from carposporophytic plants collected at Media Luna Reef. Carpospore release was initiated 24 hours after isolation of the carposporophytes, and release occurred *en masse*. Released carpospores measured 20-22 μm (21.1 ± 0.9 SD μm, n = 10) in diameter (Fig. 12a). Prior to the first division, spores enlarged to 33-35 μm in diameter (Fig. 12b). The first division resulted in two nearly equally-sized cells (Fig. 12c), one of which (the

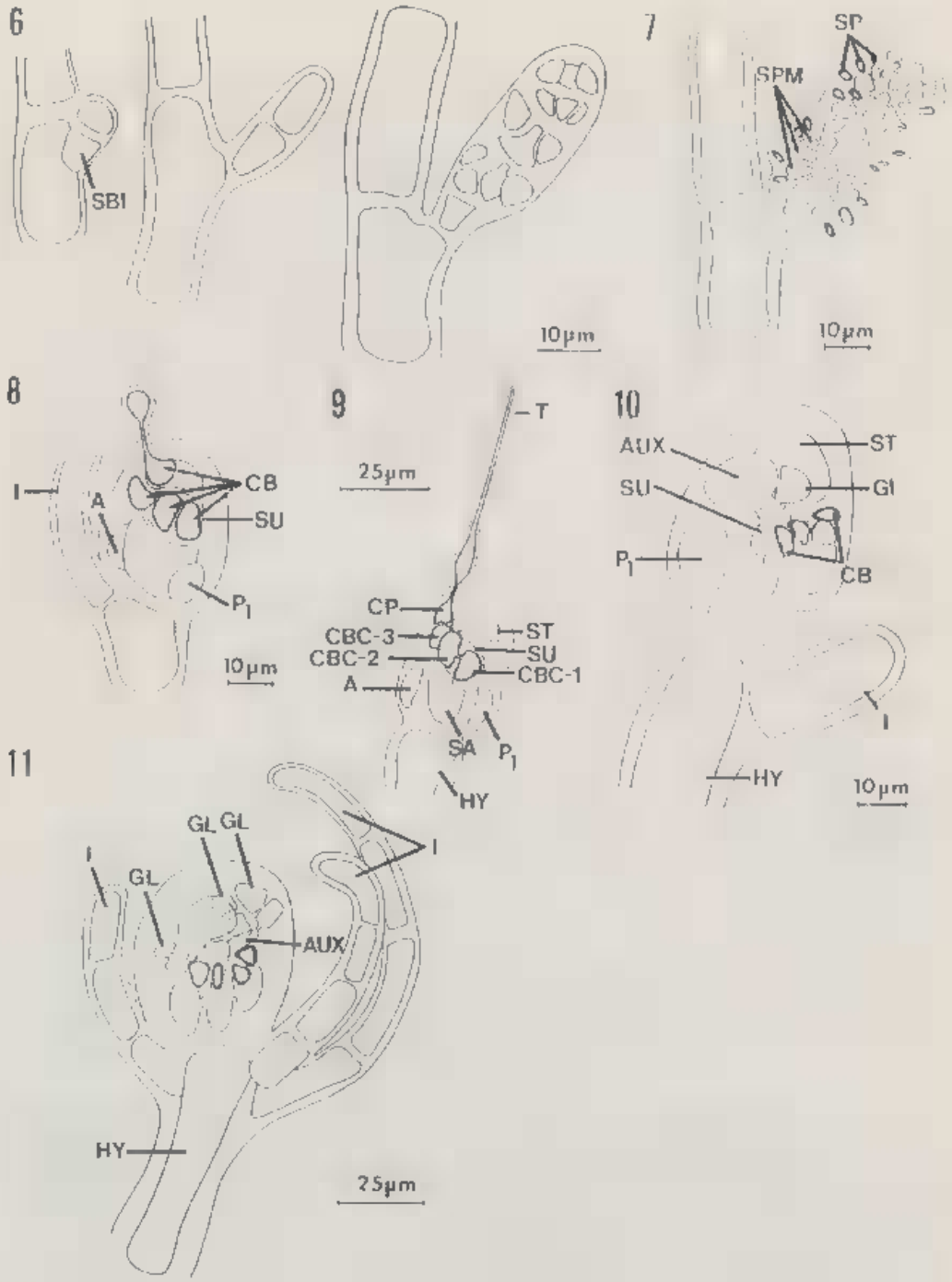

Fig. 6-11: Cultured *Pleonosporium caribaeum*. Fig. 6a, b, c. Development of spermatangial fascicles. Fig. 7. Mature spermatangial fascicle. Fig. 8. Young procarp; the hypogenous cell has initiated the development of the first involucral filament. Fig. 9. Fully developed procarp. The trichogyne is enlarged near the base. Fig. 10. Carposporophyte development: early post-fertilization showing the auxiliary cell and gonimoblast initial. Fig. 11. Carposporophyte development: The auxiliary cell has developed several gonimolobes. Remnants of the carpogonial branch are still evident. A = apical cell; AUX = auxiliary cell; CB = carpogonial branch; CBC = carpogonial branch cell; CP = carpogonium; GI = gonimoblast initial; GL = gonimolobes; HY = hypogenous cell; I = involucral filament; P_1 = periaxial cell; SA = subapical cell; SBI = spermatangial branch initial; SP = spermatangium; SPM = spermatangial mother cell; ST = sterile cell; SU = support cell.

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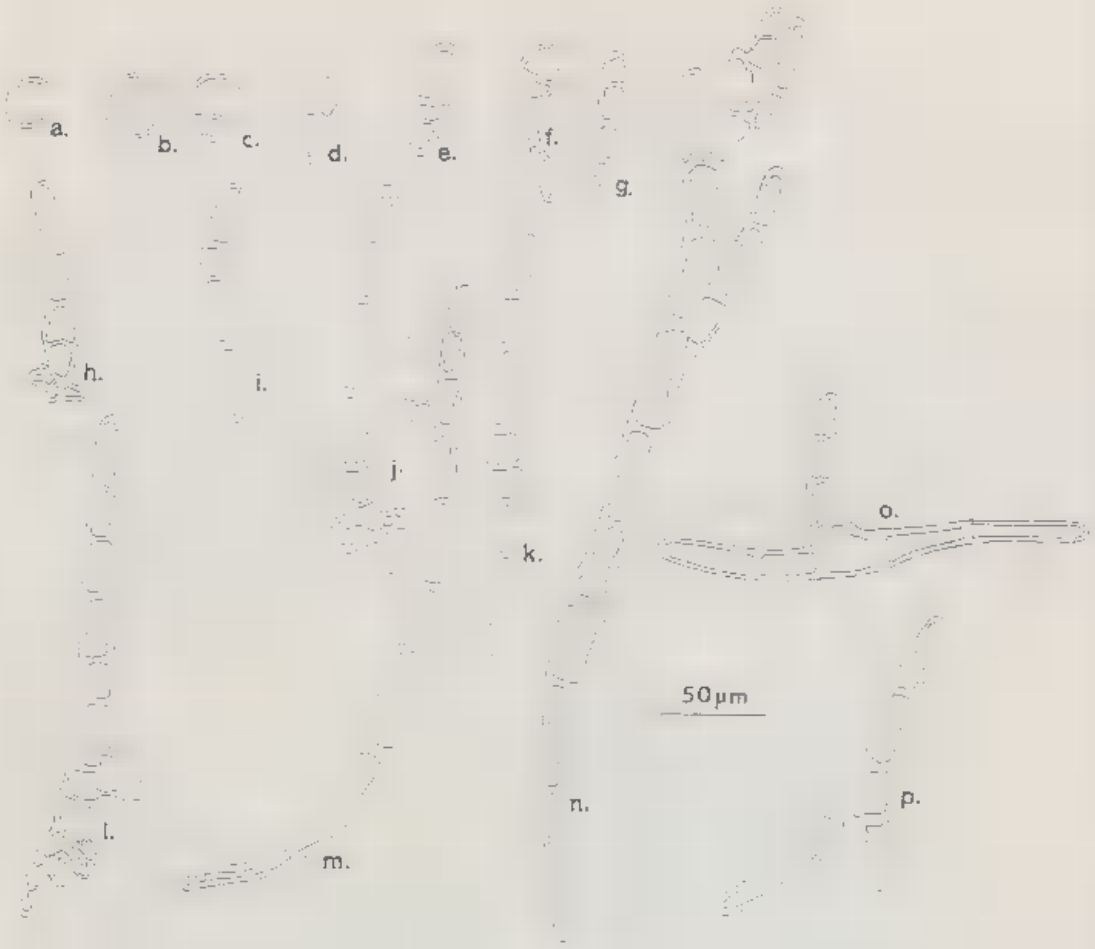

Fig. 12: *Pleonosporium caribaeum*. Fig. 12a-p. Tetrasporophyte development in culture.

rhizoidal pole) elongated and became wedge shaped (Fig. 12d). The rhizoidal pole normally developed into a long branched or simple rhizoidal filament (Fig. 12e-g, i, k-p) or formed a basal disc (Fig. 12h, j). During early early sporeling development, the erect axis developed at a slower rate than the rhizoids. The first lateral primordium of the erect axis originated at a position two to three cells behind the apical cell when the sporeling was 8-10 days old and 7-12 cells long (Fig. 12m, n). Second order branches were observed after 12 days in culture. Occasionally some rhizoidal cells became rounded and inflated and gave rise either to normally shaped rhizoidal filaments or to erect branches. These basal inflated rhizoidal cells frequently become highly entangled and provided substantial attachment.

Tetrasporophytic plants matured to produce tetrasporangia and polysporangia within 35 to 45 days. Tetrasporangia measured 30-41 μm in diameter and polysporangia generally measured over 40 μm in diameter. Released tetraspores were 15-25 μm in diameter; they germinated and developed similarly to the carpospores. Tetrad analysis demonstrated a 1:1 segregation of sexes with all plants maturing into gametophytes. Polysporangia usually contained

spores, rarely 12. Polyspores also matured into gametophytic plants and displayed a 1:1 sexual segregation.

Gametophytic plants matured after 15 days in culture, when spermatangial clusters were first observed. Initially all cylindrical spermatangial fascicles appeared to be sessile. Ten days later spermatangial clusters appearing to be both stalked and sessile were present in the culture with the former condition being most common. Procarp initials and some young procarps were observed a week after the first appearance of mature males and fertilization took place 3 to 5 days later. Abundant carposporophytes were evident a week later. In cultured plants, not all carposporangia were released from the carposporophytes. Those remaining attached to the carposporophyte germinated *in situ*. Gross morphology of cultured plants was similar to field plants. Mixed phases were never observed in plants in culture or in the field.

Habitat and Reproductive Periodicity

Plants were usually epiphytic on coarse algae or rarely found on coral and shell fragments. Sterile plants were collected irregularly throughout the year. Gametophytic and sporophytic plants were collected during May, June and July.

DISCUSSION

It would appear that the type of meiosporangia and the presence of "sessile" vs. "stalked" spermatangial fascicles in *P. caribaeum* (as well as other members of the genus) are variable characters, probably varying in response to age or environmental conditions. Specimens of *P. caribaeum* from Japan (Itono, 1971, 1977), South Africa (Norris, 1985) and Puerto Rico are now known to form tetrasporangia and polysporangia, whereas those from Korea (Kim & Lee, 1988) form only polysporangia. Presence of pedicellate polysporangia is reported for the first time in *P. caribaeum*. Pedicellate polysporangia were less common than sessile sporangia but both types occurred in cultured and field collected plants. Presence of secondary polysporangial primordia on pedicels of *Pleonosporium* has also been observed in *Pleonosporium pedicellatum* Lindstrom et Wynne (Lindstrom & Wynne, 1982). In *P. caribaeum* these were never observed to mature into functional sporangia. Post fertilization events in Caribbean *P. caribaeum* did not differ from that reported by Norris (1985) and Kim & Lee (1988).

In the present investigation, cultured male gametophytes produced "sessile" spermatangial structures that with age appeared to be stalked, although after 15 days (but prior to 45 days) both conditions were evident. In *Pleonosporium caribaeum*, the presence of a subtending stalk is the result of elongation and the degree of development of spermatangial mother cells and spermatangia on the proximal cell of the fertile male axes. Thus, the distinction of spermatangial fascicles being pedicellate or sessile is an artificial one and only refers to degree of development. Although this character has generally been regarded as taxonomically important in *Pleonosporium* (Joly, 1957; Itono, 1971, 1977), its value is questionable.

Two other species formerly assigned to *Mesothamnion*, *Pleonosporium boergesenii* (Joly) R.E. Norris and *P. yagii* (Yamada) R.E. Norris, possess both tetrasporangia and polysporangia (Norris, 1985). *Pleonosporium boergesenii* is distinguished from *P. caribaeum* in possessing "sessile" spermatangial fascicles

(Joly, 1957); however, Ardré *et al.* (1982), upon examination of syntype specimens, demonstrated the presence of both "sessile" and "stalked" male structures in *P. boergesenii*. Schneider (1975) also reported both "sessile" and "stalked" spermatangial fascicles in North Carolina plants assigned to *P. beorgesenii*. It is very probable that *P. boergesenii* is not different from *P. caribaeum* since the character used in separating the species is artificial. According to Norris (1985), separation of *P. yagii* from *P. caribaeum* is probably also unjustified.

Wynne (1985) listed six species of *Pleonosporium* from the tropical and subtropical western Atlantic. Among these, *P. caribaeum, P. boergesenii* and *P. polystichum* Oliveira (Oliveira, 1969) possess a radial branch arrangement. While distinction between the first two is unclear, *P. polystichum* can be distinguished from *P. caribaeum* by its larger cell and polysporangia size. *Pleonosporium polystichum* has polysporangia which are 68-69 μm in diameter while those of *P. caribaeum* are approximately 40-50 μm in diameter.

Pleonosporium caribaeum is a widely distributed species, having been reported from: Bahamas (Howe, 1920); Canary Islands (Afonso-Carillo & Gil-Rodriguez, 1980); France (Ardré *et al.*, 1982); Japan (Itono, 1971); Korea (Kim & Lee, 1988); Martinique (Hamel & Hamel-Joukov, 1931); South Africa (Norris, 1985; Stegenga, 1986); U.S. Virgin Islands (Børgesen, 1917) and Viet-Nam (Dawson, 1954). The first report of *P. caribaeum* from Puerto Rico was by Diaz-Piferrer (1963) based on material collected in Guánica. On examination of this material (*MDP-DB-1584*), all plants were identified ■ *Aglaothamnion boergesenii* (Aponte et Ballantine) L'Hardy-Halos et Rueness. One other specimen in MSM (*MSM-6342*) identified as *M. caribaeum* is also a species of *Callithamnion*. This report constitutes the first verifiable record of *P. caribaeum* for Puerto Rico.

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